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DOCKET NO. 52510

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: The Salk Institute for Biological Studies)
Patent No.: 4,244,946)
Issue Date: January 13, 1981)
Title: WATER-SOLUBLE PEPTIDES AFFECTING GONADAL FUNCTION)

APPLICATION FOR EXTENSION OF THE TERM OF A PATENT

Box Pat. Ext.
Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

Pursuant to 35 U.S.C. §156, The Salk Institute for Biological Studies (SALK) respectfully requests extension of the term of the above-identified U.S. patent, hereinafter "said Patent". Pursuant to 37 C.F.R. §1.740, the following information is submitted:

1. A complete identification of the active ingredient of the approved therapeutic drug product is set forth as follows: a nonapeptide analog of human Gonadotropin Releasing Hormone (variously referred to as GnRH, LRF and LH-RH) in the form of its acetate salt, more specifically, the C-terminally ethylamidated D-His^6(im-Bzl) analog thereof, variously referred to as [D-His^6(im-Bzl), Pro^9-NEt]-GnRH (or LRF) and by the Adopted Name histrelin acetate; the structural formula of the nonapeptide is pGlu-His-Trp-Ser-Tyr-D-His(im-Bzl)-Leu-Arg-Pro-$NHCH_2CH_3$ and its empirical formula is

$C_{66}H_{86}N_{18}O_{12}$. The chemical name of histrelin acetate is: 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-N^t-benzyl-D-histidyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide acetate (salt).

2. The regulatory review occurred under the Federal Food, Drug and Cosmetic Act, §505 (21 U.S.C. §355).

3. The product received approval for its first permitted commercial marketing on December 24, 1991, under the provisions of the Federal Food, Drug and Cosmetic Act, as administered by the Food and Drug Administration (FDA) under which the applicable regulatory review period occurred.

4. The human therapeutic drug product which was approved contains [D-His^6(im-Bzl), Pro^9-NEt]-GnRH (histrelin acetate) as the only active ingredient, and this active ingredient was not earlier approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act.

5. This application is being submitted within the sixty-day period permitted for submission under 37 C.F.R. §1.720(f), the date of the last day on which such application could be submitted being February 21, 1992.

6. U.S. Patent No. 4,244,946 (said Patent) is the patent for which an extension is being sought, which issued on January 13, 1981, to SALK on an application filed in the names of Jean E.F. Rivier et al.

7. Enclosed as Exhibit A is a copy of said Patent for which an extension is being sought.

8. No reexamination certificate or certificate of correction has issued in said Patent. No maintenance fees were due because the filing date of said Patent was prior to December 12, 1980. No terminal or other disclaimer has been filed.

9. Said Patent claims both the approved product and a method of using that product, and as set forth immediately herebelow, Claims 1, 3, 4 and 6 would read upon the approved product and the intended method of using the approved product.

1. A compound selected from the class defined by the formulae:

p-Glu-His-Trp-Ser-Tyr-D-His(im-Bzl)-Leu-Arg-R and its nontoxic salts, and

X^1-p-Glu-His(X^2)-Trp-Ser(X^3)-Tyr(X^4)-D-His(im-Bzl)-Leu-Arg(X^5)-Pro-X^6

wherein R is selected from the group consisting of

Pro-Gly-NH_2 and Pro-NH-CH_2-CH_3;

X^1 is either hydrogen or an α-amino protecting group;

X^2 is a protecting group for the imidazole nitrogen atom selected from the group consisting of Tos, benzyl, trityl, 2,2,2-trifluoro-1-benzyloxycarbonyl-aminoethyl, 2,2,2-trifluoro-ltert-butyloxycarbony-laminoethyl and 2,4-dinitrothiophenyl;

X_3 is a protecting group for the alcoholic hydroxyl group of Ser selected from the group consisting of acetyl, benzoyl, tetrahydropyranyl, tert-butyl, trityl, benzyl and 2,6-dichlorobenzyl;

X^4 is a protecting group for the phenolic hydroxyl group of Tyr selected from the group consisting of tetrahydropyranyl, tert-butyl, trityl, benzyl, benzyloxycarbonyl, 4-bromobenzyloxycarbonyl and 2,6-dichlorobenzyl;

X^5 is a protecting group for the nitrogen atoms of Arg selected from the group consisting of nitro, Tos, benzyloxycarbonyl, adamantyloxycarbonyl, and BOC, or is hydrogen; and

X^6 is selected from the group consisting of dimethylamine, alkylamine of 1 to 5 carbon atoms, phenethylamine, O-CH_2-[resin support], Gly-O-CH_2-[resin support], and Gly-NH[resin support].

3. A compound in accordance with claim 1 wherein R is Pro-NH-CH_2-CH_3.

The active ingredient [D-His^6(im-Bzl), Pro^9-NEt]-GnRH is the 9-residue synthetic peptide defined in the formula when R is Pro-$NHCH_2CH_3$, which is in the form of the nontoxic acetate salt.

* * * * *

4. A method for regulating fertility and the production of gonadotropins and sex steroids in male and female mammalians comprising administering an effective amount of a peptide having the formula:

p-Glu-His-Trp-Ser-Tyr-D-His(im-Bzl)-Leu-Arg-R,

wherein R is selected from the group consisting of Pro-Gly-NH_2 and Pro-NH-CH_2-CH_3, or a nontoxic salt thereof.

6. A method in accordance with Claim 4 wherein R is Pro-NH-CH_2-CH_3.

The 9-residue peptide product is approved for administration to humans for the treatment of precocious puberty by regulating the levels of gonadotropic hormones and sex steroids in males and females.

10. A new drug application (NDA) for the therapeutic use of the human drug product histrelin acetate to treat precocious puberty (NDA 19-836) having an effective date of May 19, 1989, was filed by a licensee, Ortho Pharmaceutical Corporation (hereinafter "Ortho"), under a License Agreement that was effective 30 September 1980, between SALK and Ortho, which drug product was referred to by the Adopted Name histrelin acetate and may also have been referred to by Ortho's trademark Supprelin during the course of the NDA. Number 19-836 was assigned to the NDA, and ownership of the NDA was subsequently transferred to the R.W. Johnson Pharmaceutical Research Institute (PRI), a division of Ortho. The NDA was approved on December 24, 1991.

The testing which supported Ortho's NDA submission was carried out by several independent investigators sponsored by Ortho, with William Crowley, M.D. and Ora Pescovitz, M.D. being the two principal investigators. Ortho had earlier obtained related IND No. 23,307 for a different use of the drug product, i.e. to allow investigation with respect to the use of histrelin for the treatment of endometriosis.

Dr. William Crowley, of Boston, Massachusetts, worked under his IND No. 13,353 which originally was issued to permit the use of GnRH itself, and then another GnRH analog, for the treatment of precocious puberty; it was subsequently modified in response to Dr. Crowley's letter request to the FDA dated December 24, 1984, to permit the administration of histrelin, following Ortho's earlier authorizing Dr. Crowley to cross-refer to their IND 23,307 for purposes of chemistry, toxicology and the like. Dr. Crowley's IND continued to the date of submission of the NDA and is believed to be still continuing.

An IND submission for therapeutic use of histrelin to treat precocious puberty was submitted by Dr. Ora Pescovitz on about November 6, 1985, after she had earlier been authorized by Ortho to cross-refer to Ortho's related IND. It was received by the FDA on about November 15, 1985, and assigned IND No. 27,427. It continued until the filing date of the NDA submission and is still continuing.

Additional testing sponsored by Ortho was carried out by other independent investigators, including Dr. Inese Beitins, Dr. Sam Yen, Drs. Gordon Cutler and Judy Ross, and Dr. J. Germak, who also worked under their own INDs. For example, IND No. 24,587 was obtained by Dr. Inese Beitins. These sponsored investigators were also given permission by Ortho to refer to Ortho's IND No. 23,307.

Ortho has approved of the submission of this application for extension of U.S. patent term.

11. A brief description of the significant activities undertaken by and on behalf of Ortho, the marketing applicant, during the applicable regulatory review period with respect to the product histrelin acetate (Supprelin), including the appropriate portions of IND periods for therapeutic use of the product for treatment of precocious puberty preceding the filing of the NDA submission, and the significant dates applicable to such activities is set forth hereinafter as follows:

DATE	SUBJECT
Dec. 24, 1984	Letter to Carlos Schaffenburg of FDA from William Crowley, M.D. requesting that his original IND No. 13,353 be modified to permit administration of histrelin
Jan. 1985	First patients treated with histrelin by Dr. Crowley under modified IND
Nov. 6, 1985	IND Submission by Dr. Ora Pescovitz
Nov. 15, 1985	Pescovitz IND received by FDA and assigned No. 27,427
Nov. 18, 1985	FDA acknowledges receipt and reports Pescovitz IND No.
Jan. 21, 1986	FDA sends guidelines to Pescovitz re test procedures
Aug. 31, 1988	NDA submitted by Ortho
Sept. 9, 1988	FDA acknowledgement of NDA submission-NDA 19-836
Nov. 4, 1988	Information for clinical studies requested by Dr. Robert Young of FDA
Nov. 4, 1988	FDA letter stating that NDA is not acceptable for filing and that the provisional filing date will be 11/6/88 and not 9/9/88

Nov. 23, 1988	Acknowledgement of FDA's letter of 11/4/88 and confirmation that request for reconsideration of refusal will be filed
Dec. 15, 1988	Response to FDA letter of 11/4/88 requesting reconsideration and supplying supporting information
Feb. 28, 1989	FDA response to Ortho letter of 12/15/88 - will accept application for filing under certain conditions
May 19, 1989	Resubmission of NDA 19-836
May 31, 1989	FDA acknowledgement of refiling of NDA. Indication that filing date will be accorded unless found not acceptable by 7/22/89
June 5, 1989	Transfer of NDA ownership and responsibility to PRI
June 5, 1989	Acceptance of NDA ownership and responsibility by PRI
Aug. 15, 1989	Submitting reference to the fact that ovariectomizing pregnant rats prior to delivery causes dystocia - "The Vertebrate Ovary", per request of Dr. Raheja, reviewing pharmacologist.
Aug. 30, 1989	Final reports for mouse and rat carcinogenicity studies per agreement with FDA - DS 6034/A 11,523 and DS 6033/A 11,549
Sept. 5, 1989	Revised Form 356H to reflect the appropriate divisional site where NDA is now located, i.e at PRI, Div. of Ortho Pharmaceutical Corp.
Sept. 19, 1989	FDA letter stating that drug safety reports submitted 8/30/89 are a major amendment - 90 additional days added for review
Oct. 6, 1989	FDA letter requesting additional information after preliminary review of application
Nov. 9, 1989	Preliminary response to FDA letter of 10/6/89

Nov. 30, 1989	Submitting replacement pages for report DS 6034/A 11,523, and copies of referenced articles number 15 and 16 as requested by Mr. P. Vaccari
Dec. 6, 1989	Advising division that requested computer data on computer tapes and disks for reports earlier submitted on 8/30/89 are being submitted directly to Dr. W. Fairweather, reviewing statistician
Dec. 15, 1989	Submitting the Bayley-Pinneau Height Prediction Tables and the Tanner-Whitehouse Standards for height per request of Dr. J. Fourcroy, reviewing Medical Officer
Jan. 5, 1990	Submitting MR 2461/A 11,730 Histrelin for Injection - 4 Month NDA Safety Update
Jan. 9, 1990	Response to FDA letter of 10/6/89, supplying additional information re the drug substance, drug product and labeling
Jan. 29, 1990	Advising FDA that the trademark for histrelin is SUPPRELIN and Ortho commits to deleting the number in connection with the name
Feb. 26, 1990	FDA letter advising that submission of 1/9/90 is considered a major amendment - 60 additional days added for review
March 13, 1990	Revised "Carcinogenesis, Mutagenesis and Impairment of Fertility" section of package insert per request of P. Vaccari
March 14, 1990	FDA letter with comments and requests for additional information and stating that proposed trademark SUPPRELIN should be changed to conform to requirements
March 22, 1990	Correction of typo error in the draft physicians' insert submitted 3/13/90

April 9, 1990	Response to FDA letter of 3/14/90, requesting reconsideration re proposed trademark
April 24, 1990	Medical abstracts, protocols and CVs for two controlled studies conducted by Dr. W. Crowley, and indicating that CRFs will be sent within next few days
April 26, 1990	CRFs for Dr. Crowley's study OHN101 and protocol and CVs for all investigators and co-investigators for studies by Drs. Pescovitz, Beitins, Cutler, Ross, and Yen
May 15, 1990	CRFs for Dr. Cutler's study as requested by Dr. Young
May 18, 1990	Submitted amended drug substance specification 17070-DS-J to include a residual solvent test for methanol and acetonitrile, and copy of method SOLV-X-1. Ortho commits to making all requested changes in labeling other than deleting tradename SUPPRELIN
July 31, 1990	Submits draft labeling using tradename SUPPRELIN and including all of the revisions requested in FDA letters of 10/6/89 and 3/14/90
Aug. 3, 1990	Four revised copies of the draft physician insert submitted 7/31/90
Aug. 10, 1990	Ortho provides for Hazleton Laboratories America, Inc. to serve as a contract laboratory to conduct pyrogen testing for product release and stability
Aug. 30, 1990	Draft physician insert with additional revisions per Pharmacology review, and response to Dr. Raheja's request for clarification of incidence of pituitary tumors in the control group of male rats in two year carcinogenicity study
Sept. 6, 1990	Revised draft labeling with changes requested by Dr. Raheja by phone on 9/6/90

Oct. 11, 1990	Withdrawing Ortho Pharmaceutical Inc., Manati as a packaging facility, as requested by Dr. Helen Davies reviewing chemist
Nov. 26, 1990	Responses to requests for information re Human Pharmacology section of NDA and computer disk in WordPerfect 5.0 with this information
Dec. 4, 1990	Final draft physician insert and patient information brochure with all revisions discussed with Lois Simmons, Consumer Safety Officer of FDA--information to serve as a final Safety Update
Dec. 6, 1990	Resubmission of patient information brochure including paragraph originally omitted in submission of 12/4/90
Dec. 6, 1990	Submitted responses for information requested by Mr. John Hunt, reviewing biopharmaceutics officer
Jan. 18, 1991	FDA letter advising that Methods Validation is being initiated in FDA labs - prepared two sets of the samples for Methods Validation
Nov. 13, 1991	Provided information requested in 11/8/91 telephone message from Dr. Fourcroy. Response pertained to correlation of medication, age of child, growth parameters and pituitary hormones for subjects in Dr. Crowley's clinical studies
Nov. 26, 1991	Responded to telephone request of the week of 11/18/91 regarding a revised package insert, an explanation of the patient population, and an update of adverse events
Dec. 9, 1991	Presented justification why Ortho should not revise package insert as previously requested; submitted a copy of most recent insert

Dec. 11, 1991	Responded to request of 12/11/91 that Ortho make minor revisions to the Precautions and How Supplied sections of physicians' package insert; submitted requested revisions to physicians' insert in draft form
Dec. 20, 1991	Responded to requests of 12/18/91 and 12/19/91 that Ortho make further revisions to the physicians' insert and patient information sheet; submitted requested revisions on the physicians' insert and patient information sheet in draft form
Dec. 23, 1991	Responded to request of 12/20/91 that Ortho still further revise physicians' insert and patient information sheet; submitted requested changes in draft form
Dec. 24, 1991	FDA issues approval letter of NDA 19-836

Applicant has been unable to obtain additional data from the individual investigators sponsored by Ortho concerning precise dates of specific actions which occurred during the various IND periods, but believes the information provided herein relating to the individual investigators is accurate and should be adequate for review by FDA. Applicant stands willing to supplement this application in any way, if requested, should further information of this type be needed.

12. It is the opinion of the applicant that said Patent is eligible for an extension under 35 U.S.C. §156. The initial portions of the generally concurrent Crowley and Pescovitz IND periods should properly be linked to the NDA period in this case, and it is believed that the potential extension would nominally be for a term of 1752 days from the present expiration date of said Patent, i.e. January 13, 1998. This extension is calculated on the basis of being equal to the time period occupied by the initial portions of the terms of the Crowley IND and the Pescovitz IND which preceded the effective NDA filing, i.e. from December 24, 1984, through May 19, 1989, a period of 1606 days, which period is reduced by one-half, i.e. to 803 days, pursuant to 35 U.S.C. §156(c)(2), plus the NDA review period, i.e. from May 19, 1989, through December 24, 1991, a period of 949 days. Thus, in such instance said Patent should be extended for a period of 1752 days, i.e. 4 years and 291 days, to expire on October 31, 2002, if there are no deductions for failure to act with diligence during either of the 2 periods. It appears that the limitation of the period of extension to five (5) years by 35 U.S.C. §156(g)(6)(B) would not be of consequence in this case.

13. Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to be made relative to this application for extension.

14. The undersigned declares that he:

(1) is an officer of the corporate owner of said Patent;

(2) has reviewed and understands the contents of this application being submitted herewith;

(3) believes said Patent is subject to extension pursuant to 35 U.S.C. 156 and 37 C.F.R. §1.710,

because it claims a human drug product subject to regulation under the Federal Food, Drug and Cosmetic Act;

(4) believes an extension of the length claimed is fully justified under 35 U.S.C. 156 and the applicable regulations, including 37 C.F.R. §1.775; and

(5) believes said Patent for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 37 C.F.R. §1.720 because:

(a) said Patent claims a human drug product as defined in the Federal Food, Drug and Cosmetic Act and a method for using that product.

(b) the term of said Patent has never been previously extended;

(c) this application for extension is being appropriately submitted in compliance with 37 C.F.R. §1.740;

(d) the approved product has been subjected to a regulatory review period as defined in 35 U.S.C. 156(g) before its commercial marketing or use;

(e) the product has received permission for commercial marketing or use and the permission for such commercial marketing or use of the product is the first-received permission for commercial marketing or use under the provision of law under which the applicable regulatory review occurred;

(f) the application is being submitted within the sixty day period beginning on the date the product first received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred;

(g) the term of said Patent has not expired before the submission of this application; and

(h) no other patent term has been extended for the same regulatory review period for the approved product.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent concerned.

I hereby appoint the following attorneys and agents, with full power of substitution and revocation, to prosecute this application this application and to transact all business in the United States Patent and Trademark Office connected therewith and request that all correspondence and telephone calls in respect to this application be directed to FITCH, EVEN, TABIN & FLANNERY, Suite 900, 135 South LaSalle Street, Chicago, Illinois 60603, Telephone Nos. (312) 372-7842 or (619) 552-1311:

Attorney	Reg.No.	Attorney	Reg.No.
Morgan L. Fitch, Jr.	17,023	James J. Hamill	19,958
Francis A. Even	16,880	Phillip H. Watt	25,939
Julius Tabin	16,754	Timothy E. Levstik	30,192
John F. Flannery	19,759	Joseph E. Shipley	31,137
Robert B. Jones	20,135	Bryant R. Gold	29,715
James J. Schumann	20,856	Stephanie L. Seidman	33,779
R. Steven Pinkstaff	20,448	Christine M. Bellas	34,122
		Michael A. Kagan	33,188

Full name of Officer of
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Officer's Signature: Douglas D. Busch

Date: February 20, 1992

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EXHIBIT A

United States Patent [19]

Rivier et al.

[11] **4,244,946**

[45] **Jan. 13, 1981**

[54] **WATER-SOLUBLE PEPTIDES AFFECTING GONADAL FUNCTION**

[75] Inventors: **Jean E. F. Rivier; Wylie W. Vale, Jr.,** both of La Jolla, Calif.

[73] Assignee: **The Salk Institute for Biological Studies,** San Diego, Calif.

[21] Appl. No.: **47,026**

[22] Filed: **Jun. 11, 1979**

[51] **Int. Cl.**[3] **A61K 37/00; C07C 103/52**

[52] **U.S. Cl.** ..... **424/177; 260/112.5 LH**

[58] **Field of Search** ..... 424/177; 260/112.5 LH

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,010,125	3/1977	Schally et al.	260/112.5 LH
4,018,726	4/1977	Schally et al.	260/112.5 LH
4,034,082	7/1977	Johnson et al.	424/177
4,086,219	4/1978	Wittle et al.	260/112.5 LH

OTHER PUBLICATIONS

J. Rivier, et al., "Peptides", 1976, pp. 427–451.

Primary Examiner—Delbert R. Phillips
Attorney, Agent, or Firm—Fitch, Even, Tabin, Flannery & Welsh

[57] **ABSTRACT**

[im-Bzl D-His^6]LRF and [D-His^6(im-Bzl), Pro^9-NEt]LRF exhibit hydrophillicity comparable to that of LRF and act as superagonists exhibiting potencies, respectively, about 12 and more than 200 times that of LRF. The peptides or their nontoxic salts can be administered by intravenous subcutaneous, sublingual, oral, intravaginal, intranasal or rectal routes. The peptides can be used to regulate fertility in male and female mammals, including human beings.

6 Claims, No Drawings

WATER-SOLUBLE PEPTIDES AFFECTING GONADAL FUNCTION

The invention described herein was made in the course of work under a grant or award from the Department of Health, Education, and Welfare.

The present invention relates to peptides which influence the release of gonadotropins by the pituitary gland in mammalians, including humans. More particularly, the present invention is directed to peptides which when administered acutely to mammalians exhibit increased potency in releasing gonadotropins, which subsequently cause the release of the steroidal hormones, progesterone, testosterone and estrogens.

The pituitary gland is attached to a stalk to the region in the base of the brain known as the hypothalamus and has two principal lobes, the anterior lobe and the posterior lobe. The posterior lobe of the pituitary gland stores and passes onto the general circulation system two hormones manufactured in the hypothalamus, i.e., vasopressin and oxytocin. The anterior lobe of the pituitary gland secretes a number of hormones, which are complex protein or glycoprotein molecules, that travel through the blood stream to various organs and which, in turn, stimulate the secretion into the blood stream of other hormones from the peripheral organs. In particular, follicle stimulating hormone (FSH) and luteinizing hormone (LH), sometimes referred to as gonadotropins or gonadotropic hormones, are released by the pituitary gland. These hormones, in combination, regulate the functioning of the gonads to produce testosterone in the testes and progesterone and estrogen in the ovaries, and also regulate the production and maturation of gametes.

The release of a hormone by the anterior lobe of the pituitary gland usually requires a prior release of another class of hormones produced by the hypothalamus. Such a hypothalamic hormone acts as a factor that triggers the release of the gonadotropic hormones, particularly luteinizing hormone (LH). The particular hypothalamic hormone which acts as a releasing factor for the gonadotropins LH and FSH is referred to herein as LRF, wherein RF stands for "releasing factor" and L signifies that one hormone released is LH. LRF has been isolated, identified and synthesized.

It has been demonstrated that some female mammalians who have no ovulatory cycle and who show no pituitary or ovarian defect begin to secrete normal amounts of the gonadotropins LH and FSH after the administration of LRF. Such administration of LRF is suitable for the treatment of those cases of infertility where the functional defect resides in the hypothalamus. Ovulation can also be induced in female mammalians by the administration of LRF; however, the dosage level of LRF required to influence ovulation may sometimes be high. Recent reports have also indicated that the administration of large and frequent dosages of LRF actually inhibit gonadal function in female and male rats by desensitization of the pituitary and gonads and subsequent disruption of the hormonal network. For this reason, LRF and analogs of LRF which are more potent than LRF to promote release of LH have been investigated for potential use as a contraceptive. The principal disadvantage to the use of these peptides as a potential contraceptive is, of course, the requirement for large and frequent dosages. It would be desirable to provide peptides which are many times more potent than LRF in promoting the secretion of LH.

Accordingly, it is a principal object of the present invention to provide peptides which exhibit a very high potency to cause the release of gonadotropins in mammalians, including humans. Another object of the present invention is to provide such potent peptides which influence the release of steroids by the gonads of male and female mammalians, including humans, and which have properties which favorably affect their administration. A further object of the present invention is to provide peptides which have a more potent effect than LRF on the reproduction processes of mammalians, including humans. These and other objects of the present invention will become more apparent from the following detailed description.

Generally, in accordance with the present invention, LRF agonists have been synthesized which have an enhanced potency to cause the secretion of gonadotropins by the pituitary gland of mammalians, including humans, and which peptides also can cause inhibition of the reproductive functions in both males and females, such as delay of puberty, interruption of pregnancy, decrease in sexual organ weights and steroid production, and disrupted spermogenesis. The peptides of the present invention are characterized by the substitution of (im-Bzl) D-His in the 6-position of LRF or an LRF analog.

LRF has been characterized as a decapeptide having the following structure:

p-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

Peptides are compounds which contain two or more amino acids in which the carboxyl group of one acid is linked to the amino group of the other acid. The formula for LRF, as represented above, is in accordance with conventional representation of peptides where the amino group appears to the left and the carboxyl group to the right. The position of the amino groups is identified by numbering the amino groups from left to right. In the case of LRF, the hydroxyl portion of the carboxyl group at the right-hand end has been replaced with an amino group (NH_2), to give an amide function. The abbreviations for the individual amino acid groups above are conventional and are based on the trivial name of the amino acid: where p-Glu is pyroglutamic acid, His is histidine, Trp is tryptophan, Ser is serine, Tyr is tyrosine, Gly is glycine, Leu is Leucine, Arg is arginine and Pro is proline. Except for glycine, amino acid residues in the peptides of the invention should be understood to be of the L-configuration unless noted otherwise.

It is known that the substitution of a D-amino acid (for example D-Trp) for Gly in the 6-position of the LRF decapeptide provides a peptide material having from about 10 to 30 times greater potency than does LRF to effect the release of luteinizing hormone and other gonadotropins by the pituitary gland of mammalians. The releasing effect is obtained when the substituted peptide is introduced into the blood stream of a mammalian. The desired peptides are not significantly different in their hydrophilicity from LRF, whereas other potent LRF analogs are significantly less hydrophilic, and this will provide opportunities for administration in various ways including those most suitable for peptides having a longer duration of effect.

In accordance with the present invention, peptides have been synthesized which are highly potent to release gonadotropins and are represented by the following formula:

p-Glu-His-Trp-Ser-Tyr-D-His(im-Bzl)-Leu-Arg-R wherein R is selected from the group consisting of Pro-Gly-NH_2 and Pro-NH-CH_2-CH_3. D-His(im-Bzl) refers to imidazole benzyl D-histidine wherein the benzyl group is attached to one of the nitrogen atoms in the imidazole ring of the histidine residue.

The peptides of the present invention having D-His-(im-Bzl) in the 6-position have greatly enhanced potency compared to other known LRF analogs which have been reported earlier, for example in U.S. Pat. Nos. 3,896,104, 3,972,859 and 4,034,082. The enhanced potency of these LRF agonists and the fact that they are substantially more hydrophilic than other analogs renders them of significant value in treating both male and female infertility and also in the inhibition of reproductive functions in both males and females as a result of long-term administration.

The peptides of the present invention are synthesized by a solid phase technique. The synthesis is preferably conducted in a stepwise manner on a chloromethylated resin when R is Pro-NH-CH_2-CH_3 and on a benzhydrylamine or a methyl-benzhydrylamine resin when R is Pro-Gly-NH_2. However, a chloromethylated resin may also be used when R is Pro-Gly-NH_2 because aminolysis of the glycine benzyl ester can be achieved using ammonia. The resin is composed of fine beads (20–70 microns in diameter) of a synthetic resin prepared by copolymerization of styrene with 1 to 2 percent divinylbenzene. For a chloromethylated resin, the benzene rings in the resin are chloromethylated in a Friedel-Crafts reaction with chloromethyl ether and stannic chloride, and the chlorine introduced is a reactive benzyl chloride. The Friedel-Crafts reaction is continued until the resin contains 0.5 to 2 millimoles of chlorine per gram of resin. The benzhydrylamine resin is prepared in accordance with the teaching of U.S. Pat. No. 4,072,688 issued Feb. 7, 1976 to Max S. Amoss et al. More recently, a paramethyl-BHS has been used, which may be obtained as generally described in U.S. Pat. No. 4,072,688 with the exception that p-toluolyl chloride is used instead of benzyl chloride in the Friedel-Crafts step. Mild conditions during HF cleavage can be used with such a resin, and as a result, a purer peptide is obtained than the equivalent one made on regular BHA.

The reagents used are hereinbelow first listed by their chemical name and their common abbreviation.

A peptide wherein R is Pro-NH-CH_2-CH_3 or Pro-Gly-NH_2, may be prepared, for example by esterifying the triethylammonium salt of α-amino protected Pro or Gly onto the chloromethylated resin by refluxing in ethanol for about 48 hours. Also possible is the use of α-amino protected Pro, potassium or cesium salts in dimethylformamide (DMF) or in dimethylsulfoxide (DMS), at temperatures ranging from 40° to 80° C. Further possible is the use of the α-amino protected Pro dissolved in DMF in combination with the chloromethylated resin in the presence of KF. After deprotection of the α-amino N-terminus and neutralization, the stepwise addition of N-protected amino acids is effected as generally taught in Monahan, et al. Biochemistry (1963) Volume 12, P. 4616–4620. The N^{α} groups may be protected by t-butoxycarbonyl (BOC), and the side chain of Arg may be protected with p-toluenesulfonyl (Tos). Benzyl ester (OBzl) may be used as a side chain protecting group for Ser and Tyr. 2–6 dichlorobenzyl may be used as the side chain protecting group for Tyr; and Tos, dinitrophenyl (Dnp) or BOC can be used as the side chain protecting group for His. pGlu may be introduced, for example, as benzyloxycarbonyl (Z) protected amino acid, or without any protection.

Such a method provides the fully protected peptidoresin, and the fully protected peptide is removed from the resin support in a suitable manner, e.g., using ammonia or by aminolysis employing dimethylamine, methylamine, ethylamine, n-propylamine, i-propylamine, butylamine, iso-butylamine, pentylamine or phenethylamine to yield a fully protected alkyl amide intermediate. As one example, cleavage of the peptide from the resin may be performed by stirring the peptidoresin (. . . Pro-O-CH_2-resin) overnight in distilled ethylamine at 0° C. in a pressure bottle. As another example, the peptidoresin (. . . Pro-Gly-O-CH_2-resin) may be treated for several days in dry methanol which is kept saturated with NH_3 by bubbling gaseous ammonia therethrough. After removal of excess ethylamine or methanolic ammonia by distillation under nitrogen or vacuum, the resin, suspended in methanol, is removed from the slurry by filtration. The resin is further washed successively with dimethylformamide (DMF), methanol, and a mixture of DMF and methanol. The recovered solution of cleaved, protected peptide is evaporated to dryness on a rotary vacuum evaporator at room temperature. The peptide is taken in a minimum amount of methanol to dissolve the peptide. The solution is added dropwise with stirring to a 200-times volume excess of dry ether. A flocculent precipitate appears which is recovered by filtration or centrifugation. The recovered precipitate is dried to provide the intermediate which is considered part of the invention.

The intermediates of the invention may be represented as:

X^1-p-Glu-His(X^2)-Trp-Ser(X^3)-Tyr(X^4)-D-His(im-Bzl)-Leu-Arg(X^5)-Pro-X^6 wherein: X^1 is either hydrogen or an α-amino protecting group of the type known to be useful in the art in the stepwise synthesis of polypeptides. Among the classes of α-amino protecting groups covered by X^1 are (1) acyl-type protecting groups, such as formyl, trifluoroacetyl, phthalyl, Tos, benzensulfonyl, nitrophenylsulfenyl, tritylsulfenyl, o-nitrophenoxyacetyl, chloroacetyl, acetyl and y-chlorobutyrul; (2) aromatic urethan-type protecting groups, e.g., benzyloxycarbonyl and substituted benzyloxycarbonyl, such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl and p-methoxybenzyloxycarbonyl; (3) aliphatic urethan protecting groups, such as BOC, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl and allyloxycarbonyl; (4) cycloalkyl urethan-type protecting groups, such as cyclopentyloxycarbonyl, adamantyloxycarbonyl and cyclohexyloxycarbonyl; (5) thiourethan-type protecting groups, such as phenylthiocarbonyl; (6) alkyl-type protecting groups, such as triphenylmethyl (trityl) and benzyl; (7) trialkylsilane groups, such as trimethylsilane. The preferred α-amino protecting group is BOC.

X^2 is a protecting group for the imidazole nitrogen atom selected from the group consisting of Tos, benzyl, trityl, 2,2,2-trifluoro-1-benzyloxycarbonylaminoethyl, 2,2,2,-trifluoro-1-tert-butyloxycarbonylaminoethyl and 2,4-dinitrothiophenyl.

X^3 is a protecting group for the alcoholic hydroxyl group of Ser and is selected from the group consisting of acetyl, benzoyl, tetrahydropyranyl, tert-butyl, trityl, benzyl and 2,6-dichlorobenzyl and preferably is benzyl.

X^4 is a protecting group for the phenolic hydroxyl group of Tyr selected from the group consisting of

tetrahydropyranyl, tert-butyl, trityl, benzyl, benzyloxycarbonyl, 4-bromobenzyloxycarbonyl and 2,6-dichlorobenzyl.

X^5 is a protecting group for the nitrogen atoms of Arg and is selected from the group consisting of nitro, Tos, benzyloxycarbonyl, adamantyloxycarbonyl; and BOC; or is hydrogen which means there are no protecting groups on the side chain nitrogen atoms of arginine.

X^6 is selected from dimethylamine, alkylamine of 1 to 5 carbon atoms, phenethylamine, O-CH_2-[resin support] or Gly-O-CH_2-[resin support] or Gly-NH [resin support].

The criterion for selecting side chain protecting groups for X^2-X^5 are that the protecting group must be stable to the reagent under the reaction conditions selected for removing the α-amino protecting group at each step of the synthesis, the protecting group must not be split off under coupling conditions and the protecting group must be removable upon completion of the synthesis of the desired amino acid sequence under reaction conditions that will not alter the peptide chain.

When the X^6 group is -O-CH_2-[resin support] or Gly-O-CH_2-[resin support], what is represented is the ester moiety of one of the many functional groups of the polystyrene resin support. When the X^6 group is Gly-NH-[resin support], an amide bond connects Gly to benzhydrylamine resin or to methyl benzhydrylamine resin.

For the preparation of a peptide wherein R is Pro-Gly-NH_2 on a benzhydrylamine resin, N-termini and side chain protecting groups as generally defined above are used for the synthesis. Coupling of the Gly residue is carried out for 1 to 5 hours in methylenechloride (CH_2Cl_2), dimethylformamide (DMF) or mixtures thereof, using a 2–5 fold excess of BOC-protected amino acid and dicylcohexylcarbodiimide (DCC) activating reagent. The first residue is attached to the benzhydrylamine resin by an amide bond. The coupling reaction throughout the synthesis is monitored by a ninhydrin test, as reported by Kaiser et al. *Anal. Biochem.* 34 (1970) 595.

Deblocking is effected by a 20-minute treatment in TFA containing 5 percent 1,2-ethanedithiol, followed by neutralization with triethylamine (Et_3N) in DMF or methylene chloride. Numerous washes with MeOH and CH_2Cl_2 follow each step. The individual amino aid residues are added sequentially to complete the peptide chain.

Deprotection of the peptides and/or cleavage of the peptide from a benzhydrylamine resin or paramethyl-BHA resin may take place at 0° C. with hydrofluoric acid (HF) or other suitable reagent. Anisole or some other appropriate scavenger, e.g., methyl anisole or thioanisole, is preferably added to the peptide prior to treatment with HF. After the removal of HF, under vacuum, the cleaved, deprotected peptide is treated with ether, filtered, extracted in dilute acetic acid, separated from the resin by filtration and lyophilized.

Purification of the peptide may be effected by ion exchange chromatography on a carboxyl methyl cellulose (CMC) column, followed by partition chromatography on a gel filtration column using the elution system: n-butanol; acetic acid; water (4:1:5; volume ratio). Sephadex G 25 may be the partition chromatography column packing, and other cation exchange, such as CM-Sephadex or counter-current distribution, can also be used for the purification.

The peptides are used at a level effective to promote ovulation in female mammals and can also be used for other pharmaceutical purposes for which LRF has heretofore been employed. Because the potency of the peptides of the invention is about 12 and 217 times that of LRF (see Table I, hereinafter) the dosage may be determined for each application on the basis of such a ratio, taking other factors such as the subject of administration into consideration. For example, a suitable dosage may be within the range of about 5 ng. (nanograms) to 10 μg. daily, per kilogram of body weight.

The peptide can be administered to mammals intravenously, subcutaneously, intramuscularly, intranasally, vaginally, orally or sublingually. The effective dosage will vary with the form of administration and the particular species of mammal to be treated. Oral administration may be in either solid or liquid form.

Because the peptides of the invention exhibit hydrophillicity comparable to that of LRF, higher concentrations can be prepared in aqueous or saline solutions which provide significant advantages in administration over the other superagonist analogs reported thus far. A most important advantage lies in the fact that such an aqueous peptide solution can be administered intranasally.

The peptide may also be prepared and administered in the form of a pharmaceutically acceptable nontoxic salt, such as an acid-addition salt, or an appropriate metal complex, e.g., with zinc, iron or the like. Illustrative of pharmaceutically acceptable non-toxic salts of peptides are hydrochloride, hydrobromide, sulfate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, and the like.

The following Examples further illustrate various features of the invention but are intended to in no way limit the scope of the invention which is defined in the appended claims.

EXAMPLE I

[im-Bzl D-His^6]-LRF having the following formula is prepared by the solid phase synthesis: p-Glu-His-Trp-Ser-Tyr-D-His(im-Bzl)-Leu-Arg-Pro-Gly-NH_2.

A paramethyl benzhydrylamine resin is used, and BOC-protected Gly is coupled to the resin over a 2-hour period in CH_2Cl_2 using a 3-fold excess of the BOC reagent and dicyclohexlcarbodiimide (DCC) as an activating reagent. This attaches the glycine residue to the benzhydrylamine residue by an amide bond.

Following the coupling of each amino acid residue, washing, deblocking and coupling of the next amino acid residue is carried out in accordance with the following schedule using an automated machine and beginning with about 5 grams of resin:

Step	Reagents and Operations	Mix Times Min.
1	CH_2Cl_2 wash 80 ml. (2 times)	3
2	Methanol (MeOH) wash 30 ml. (2 times)	3
3	CH_2Cl_2 wash 80 ml. (3 times)	3
4	50 percent trifluoroacetic acid (TFA) plus 5 percent 1,2-ethanedithiol in CH_2Cl_2 70 ml. (2 times)	10
5	CH_2Cl_2 wash 80 ml. (2 times)	3
6	Triethylamine (Et_3N) 12.5 percent in 70 ml. of CH_2Cl_2 (2 times)	5
7	MeOH wash 40 ml. (2 times)	2
8	CH_2Cl_2 wash 80 ml. (3 times)	3
9	BOC-amino acid (10 mmoles) in 30 ml. of either DMF or CH_2Cl_2.	

-continued

Step	Reagents and Operations	Mix Times Min.
	depending upon the solubility of the particular protected amino acid, (1 time) plus dicyclohexylcarbodiimide (DCC) (10 mmoles) in CH_2Cl_2	30–300
10	MeOH wash 40 ml. (2 times)	3
11	Et_3N 12.5 percent in CH_2Cl_2 70 ml. (1 time)	3
12	MeOH wash 30 ml. (2 times)	3
13	CH_2Cl_2 wash 80 ml. (2 times)	3

After step 13, an aliquot is taken for a ninhydrin test: if the test is negative, go back to step 1 for coupling of the next amino acid; if the test is positive or slightly positive, go back and repeat steps 9 through 13.

The above schedule is used for coupling of each of the amino acids of the peptide of the invention after the first amino acid has been attached. N^{α} BOC protection is used for each of the remaining amino acids throughout the synthesis. The side chain of Arg is protected with Tos. OBzl is used as a side chain protecting group for the hydroxyl group of Ser, and 2–6 dichlorobenzyl is used as the side chain protecting group for the hydroxyl group of Tyr. p-Toluenesulfonyl (Tos) is used as the side chain protecting group for His at the 2-position, but D-His(im-Bzl) does not require side-chain protection. pGlu is introduced as the benzyloxycarbonyl (Z) protected amino acid or as plain p-Glu. The following amino acids, which have low solubility in CH_2Cl_2, are coupled using DMF: BOC-Arg(Tos); BOC-Trp; Z-pGlu or pGlu; and D-His(im-Bzl).

The cleavage of the peptide from the resin and complete deprotection of the side chains with the exception of (im-Bzl) of D-His^6 takes place very readily at 0° C. with hydrofluoric acid (HF). Anisole is added as a scavenger prior to HF treatment. After the removal of HF under vacuum, the resin is extracted with 0.1% acetic acid, and the washings are lyophilized to provide a crude peptide powder.

Purification of the peptide is then effected by ion exchange chromatography on carboxymethyl cellulose (Whatman CM 32, using a step gradient of 0.125 M NH_4OAc) followed by partition chromatography in a gel filtration column using the elution system: n-Butanol; Acetic acid; Water (4:1:5--volume ratio).

[D-His^6(im-Bzl)]-LRF is judged to be homogeneous using thin layer chromatography with several different solvent systems and using reversed-phase high pressure liquid chromatography as generally taught in Rivier, "Use of Trialkyl Ammonium Phosphate (TAAP) Buffers in Reverse Phase HPLC for High Resolution and High Recovery of Peptides and Proteins", *Journal of Liquid Chromatography*, 1(3), 343–366 (1978) and employing an aqueous triethylammonium phosphate buffer plus acetonitrile as the solvent system. Amino acid analysis of the resultant, purified peptide is consistent with the formula for the prepared structure, showing substantially integer-values for each amino acid in the chain. Nuclear magnetic resonance spectra is also consistent and shows the presence of the benzyl group. The optical rotation is measured on a photoelectric polarimeter $[\alpha]_D^{22} = -26.0°$ (c = 1, 1% acetic acid).

EXAMPLE II

The LRF analog [D-His^6(im-Bzl), Pro^9-NEt]-LRF is synthesized by solid phase technique in a stepwise manner on a chloromethylated resin prepared by the copolymerization of styrene with about 1% divinylbenzene.

The triethylammonium salt of BOC-protected Pro is esterified onto the chloromethylated resin by refluxing in ethanol for about 48 hours. After deprotection and neutralization, the BOC-derivative of the next amino acid, Arg, and each successive amino acid, is added in accordance with the procedure set forth in Example I.

The fully protected peptide is removed from the resin support by aminolysis employing ethylamine to yield the fully protected alkyl amide intermediate. Cleavage of the peptide is performed by stirring the resin overnight in distilled ethylamine at 0° C. in a pressure bottle. After removal of excess ethylamine by distillation under vacuum, the resin, suspended in methanol, is removed from the slurry by filtration. The resin is further washed successively with DMF, methanol, and a mixture of DMF and methanol. The recovered solution of cleaved, protected peptide is evaporated to dryness on a rotary vacuum evaporator at room temperature. Using a minimum amount of methanol to dissolve the peptide, the solution is added dropwise to a 250-times volume excess of dry ether with stirring. A flocculent precipitate appears and is recovered by centrifugation. The recovered precipitate is dried to provide the intermediate, which is then completely deprotected using HF as earlier described.

Purification of the peptide is effected by ion exchange chromatography on a CMC column, followed by partition chromatography using the elution system: n-butanol; acetic acid; water (4:1:5—volume ratio). The partition chromatography column is Sephadex G 25.

[D-His^6(im-Bzl)Pro^9NEt]-LRF is judged to be homogeneous using thin layer chromatography and several different solvent systems, as well as by using reversed-phase high pressure liquid chromatography and an aqueous triethylammonium phosphate solution plus acetonitrile. Amino acid analysis of the resultant, purified peptide is consistent with the formula for the prepared structure, showing substantially integer-values for each amino acid in the chain. Nuclear magnetic resonance spectra is also consistent and shows the presence of the benzyl group. The optical rotation is measured on a photoelectric polarimeter $[\alpha]_D^{22} = -33.9°$ (c = 1, 1% acetic acid).

The peptides prepared in the foregoing Example I are assayed in vitro using a four-day-old primary culture of dispersed rat pituitary cells and compared with LRF. The levels of LH secreted over a 4-hour period in response to the application of peptides are assayed by specific radioimmunoassay for rat LH. The results of testing are expressed in Table I herebelow:

TABLE I

TREATMENT	NANOGRAMS OF LH SECRETED
Control	612
0.1 nM LRF	1255
0.3 nM LRF	1767
1.0 nM LRF	2167
3.0 nM LRF	2867
0.003 nM Ex. I	885
0.01 nM Ex. I	1345
0.03 nM Ex. I	2150
0.1 nM Ex. I	2225
0.3 nM Ex. I	2667

The treatment procedure is repeated using the peptide prepared in Example II and the results set forth in Table II are obtained:

TABLE II

TREATMENT	NANOGRAMS OF LH SECRETED
Control	500
0.3 nM LRF	631
1.0 nM LRF	1001
3.0 nM LRF	1496
0.003 nM Ex. II	895
0.01 nM Ex. II	1256
0.03 nM Ex. II	2008

The peptide prepared in Example I has a relative potency, compared to LRF, of 12(5.8–24)—the confidence limits being shown in the parentheses. For the peptide prepared in Example II, the relative potency is 217(57–952). Based upon these tests, it can be seen that [D-His^6(im-Bzl)]-LRF has a potency about 12 times that of LRF and that [D-His^6(im-Bzl), Pro^9-NEt]-LRF has a potency of more than 200 times that of LRF.

The effectiveness of the peptide compositions prepared in Examples I and II is also tested in vivo, and the relative agonistic potencies of peptides determined in the in vitro assays reported above correlate well with the potencies obtained from in vivo tests. Comparison of the results shows that both peptide compositions are very significantly more potent than LRF when tested in vivo.

Based upon the foregoing, the peptides of the invention can be used to regulate fertility in male and female animals and human beings. High, frequency administrations of these peptides will inhibit fertility by blocking ovulation, including premature luteolysis and terminating pregnancy in females and in inhibiting spermatogenesis in males. Lower, intermittent administrations can restore fertility in those infertile states caused by LRF deficiency and can also allow timing of ovulation in normal females. The peptides can also be employed to reduce levels of sex steroids, and thus they can be used in the management of subjects with sex hormone dependent neoplasms. As earlier mentioned, the peptides can be administered by intravenous, subcutaneous, subligual, oral, intravaginal, intranasal or rectal routes. The high water solubility of these peptides permits higher concentrations to be dissolved in physiologic solutions.

Although the invention has been described with regard to its preferred embodiments, it should be understood that changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims which are appended hereto.

Various features of the invention are emphasized in the claims which follow.

What is claimed is:

1. A compound selected from the class defined by the formulae:

p-Glu-His-Trp-Ser-Tyr-D-His(im-Bzl)-Leu-Arg-R and its nontoxic salts, and

X^1-p-Glu-His(X^2)-Trp-Ser(X^3)-Tyr(X^4)-D-His(im-Bzl)-Leu-Arg(X^5)-Pro-X^6

wherein R is selected from the group consisting of Pro-Gly-NH_2 and Pro-NH-CH_2-CH_3;

X^1 is either hydrogen or an α-amino protecting group;

X^2 is a protecting group for the imidazole nitrogen atom selected from the group consisting of Tos, benzyl, trityl, 2,2,2-trifluoro-1-benzyloxycarbonyl-aminoethyl, 2,2,2-trifluoro-1tert-butyloxycarbony-laminoethyl and 2,4-dinitrothiophenyl;

X^3 is a protecting group for the alcoholic hydroxyl group of Ser selected from the group consisting of acetyl, benzoyl, tetrahydropyranyl, tert-butyl, trityl, benzyl and 2,6-dichlorobenzyl;

X^4 is a protecting group for the phenolic hydroxyl group of Tyr selected from the group consisting of tetrahydropyranyl, tert-butyl, trityl, benzyl, benzyloxycarbonyl, 4-bromobenzyloxycarbonyl and 2,6-dichlorobenzyl;

X^5 is protecting group for the nitrogen atoms of Arg selected from the group consisting of nitro, Tos, benzyloxycarbonyl, adamantyloxycarbonyl, and BOC, or is hydrogen; and

X^6 is selected from the group consisting of dimethylamine, alkylamine of 1 to 5 carbon atoms, phenethylamine, O-CH_2-[resin support], Gly-O-CH_2-[resin support], and Gly-NH [resin support].

2. A compound in accordance with claim 1 wherein R is Pro-Gly-NH_2.

3. A compound in accordance with claim 1 wherein R is Pro-NH-CH_2-CH_3.

4. A method for regulating fertility and the production of gonadotropins and sex steroids in male and female mammalians comprising administering an effective amount of a peptide having the formula:

p-Glu-His-Trp-Ser-Tyr-D-His(im-Bzl)-Leu-Arg-R, wherein

R is selected from the group consisting of Pro-Gly-NH_2 and Pro-NH-CH_2-CH_3, or a nontoxic salt thereof.

5. A method in accordance with claim 4 wherein R is Pro-Gly-NH_2.

6. A method in accordance with claim 4 wherein R is Pro-NH-CH_2-CH_3.

* * * * *

DOCKET NO. 52510

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: The Salk Institute For Biological Studies)
Patent No.: 4,244,946)
Issue Date: January 13, 1981)
Title: WATER-SOLUBLE PEPTIDES AFFECTING GONADAL FUNCTION)

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LETTER OF TRANSMITTAL

Hon. Commissioner of Patents
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Dear Sir:

Transmitted herewith is an Application for Extension of the Term of U.S. Patent No. 4,244,946 pursuant to 35 U.S.C. §156, together with a copy of such Application that is certified to be a correct copy thereof. Further enclosed is our check in the amount of $1,000.00 to cover the fee established by 37 C.F.R. §1.20.

Should no proper payment be enclosed herewith, as by the check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the

Commissioner is authorized to charge any unpaid amount to Deposit Account No. 06-1135, this letter being provided in duplicate.

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